PHARMACOKINETICS AND DRUG DISPOSITION

Human sympathetic activation by α_2-adrenergic blockade with yohimbine: Bimodal, epistatic influence of cytochrome P450–mediated drug metabolism

Background: **α_2-Adrenergic blockade responses suggest adrenergic dysfunction in hypertension. α_2-Blockade is also used to treat autonomic dysfunction. However, pharmacokinetic determinants of yohimbine disposition are not understood.**

Methods: **We evaluated α_2-blockade with intravenous yohimbine in 172 individuals. Specific cytochrome P450 (CYP) isoform–mediated metabolism was investigated. Results were evaluated by ANOVA and by maximum likelihood analysis for bimodality of response distributions.**

Results: **Yohimbine metabolism to 11-hydroxy-yohimbine displayed greater than 1000-fold variability, with 17 individuals showing no metabolism. Nonmetabolizers differed from others in ethnicity but not in age, sex, body habitus, blood pressure, heart rate, or family history of hypertension. Bimodality of metabolism was suggested by frequency histogram, as well as maximum likelihood and cluster analysis. Among ethnic groups, subjects of European ancestry had the highest frequency of nonmetabolism. In vitro oxidation suggested that the major route of metabolism (lowest Michaelis-Menten constant and greatest intrinsic clearance) was likely via CYP2D6 to 11-hydroxy-yohimbine. In vivo genotypes at both *CYP2D6* and *CYP3A4* were necessary to predict metabolism (overall $F = 3.03$, $P = .005$); an interaction of alleles at these 2 loci (interaction $F = 3.05$, $P = .033$) suggested an epistatic effect on drug metabolism in vivo. Nonmetabolizers had greater activation of sympathetic nervous system activity. Yohimbine increased blood pressure, an effect mediated hemodynamically by elevation of cardiac output rather than systemic vascular resistance. Blood pressure and cardiac output responses did not differ by metabolizer group.**

Conclusions: **We conclude that heterogeneous, bimodally distributed yohimbine metabolism depends on common genetic variation in both *CYP2D6* and *CYP3A4* and contributes to differences in sympathetic neuronal response to α_2-blockade. These results have implications for both diagnostic and therapeutic uses of this α_2-antagonist. (Clin Pharmacol Ther 2004;76:139-53.)**

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Adrenergic receptors play crucial roles in hypertension, with particularly heritable roles for α_2-adrenergic subtypes in both human[1-11] and rodent[12-28] genetic hypertension. α_2-Blockade causes increments in sympa-

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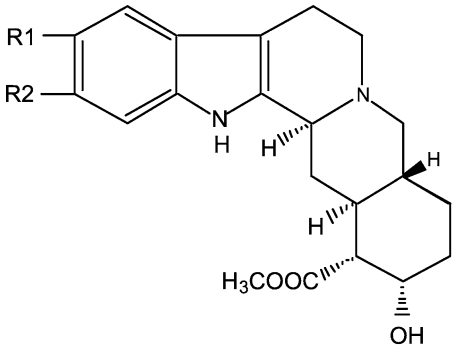

Fig 1. Chemical structure of yohimbine (R1 = R2 = H) and its 2 hydroxylated metabolites, 10-hydroxy-yohimbine (R1 = OH, R2 = H) and 11-hydroxy-yohimbine (R1 = H, R2 = OH).

thetic outflow that elevate blood pressure,[1,2,29,30] and the α_2-adrenergic antagonist yohimbine is a useful probe of α_2-adrenergic involvement or dysfunction in disease states such as hypertension.[1,2,29,30] Indeed, we found that a subset of young normotensive individuals at genetic risk of hypertension displayed exaggerated increments in catecholamine release and blood pressure after yohimbine administration,[1] suggesting that α_2-blockade might be a useful "intermediate phenotype" in the genetic analysis of the etiologically complex trait of human hypertension.[31]

Therapeutically, yohimbine has also been used to treat impotence or erectile dysfunction, orthostatic hypotension in autonomic failure, xerostomia, narcolepsy, sensorineural deafness, and depression.[32,33]

However, yohimbine itself is oxidatively metabolized to 2 hydroxylated forms (Fig 1),[34] a process not extensively characterized in diverse human populations. We tested whether there was variation in yohimbine metabolism and whether such differences influenced the response to yohimbine. Our results suggest that there is substantial interindividual variability in yohimbine metabolism, which is bimodally distributed, and that variation in metabolism influences the degree of sympathetic activation achieved by the drug.

METHODS

Subjects

We studied 172 individuals, stratified by sex (85 men and 87 women), ethnicity (73 white [European ancestry], 74 black [sub-Saharan African ancestry], and 25 other [Mexican-American or east Asian ancestry]), blood pressure (52 hypertensive and 120 normotensive), genetic risk of hypertension (162 with positive family history and 10 with negative family history), and diabetes (9 with type 2 diabetes and 163 without). Ethnicity was determined by self-identification. The subjects' usual diet was unrestricted in sodium intake. The normotensive subjects were healthy, were taking no medications, and had a diastolic blood pressure less than 90 mm Hg on at least 3 separate measurements. The hypertensive subjects had a diastolic blood pressure greater than 90 mm Hg on at least 3 separate measurements and either had never been treated or had stopped taking all antihypertensive medication for at least 3 days before the study. The hypertensive subjects had a diagnosis of essential hypertension after clinical evaluation (history, physical examination, chemistry panel, hemogram) yielded no evidence of secondary hypertension. Genetic risk (positive family history) of hypertension was defined (as previously described) by a documented diastolic blood pressure greater than 90 mm Hg or elevated blood pressure requiring medication in a parent (or sibling) before the age of 60 years.[35-38] Negative family histories were verified by contact with the subjects' parent(s) and documentation of recent normal blood pressure. Subjects with indeterminate (uncertain [eg, because of adoption]) family histories were excluded. The study protocol was approved by the Institutional Review Board of the University of California at San Diego, San Diego, Calif, and written informed consent was obtained from each subject.

Methods

The α_2-adrenergic antagonist yohimbine (as the hydrochloride salt) was purchased from the Food and Drug Division of Sigma Chemical Company (St Louis, Mo). The study was conducted under US Food and Drug Administration Investigational New Drug approval.

Experimental procedures

Subjects underwent the study in the morning after having abstained from food, cigarettes, alcohol, or caffeine since midnight. Each subject lay in the supine position with 2 heparin-lock intravenous lines (1 in each arm) in place for 30 minutes before baseline measurements were obtained.

A solution of the α_2-adrenergic antagonist yohimbine was filter-sterilized and administered intravenously as a slow 125-μg/kg bolus for 3 minutes, followed by a 1–$\mu g \cdot kg^{-1} \cdot min^{-1}$ infusion for 12 additional minutes.[1,2,29,30] Arterial blood pressures were continuously and noninvasively monitored with a finger-cuff system (Finapres; Ohmeda, Englewood, Colo) as previously described.[1,35,36] Cardiac output, heart rate, and stroke volume were noninvasively mea-

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sured by use of a thoracic impedance cardiography system (NCCOM-3; Bomed, Irvine, Calif).[1,37,38] We have previously validated the reproducibility of impedance cardiographic measurements.[36] Measurements were recorded at baseline and then at 5-minute intervals for 20 minutes after the start of infusion. A cardiac contractility index, defined as ejection velocity index normalized to thoracic fluid index, was calculated automatically from impedance data.[1,37] Blood samples were obtained at baseline (t = 0 minutes) and at the end of infusion (t = 15 minutes) for determination of levels of plasma catecholamines, as well as yohimbine and its metabolite 11-hydroxy-yohimbine. Tremor (generally fine and symmetric) and mild anxiety were common during the infusion, though transient. There were no noticeable alterations in cognition or affect.

Assays

Plasma catecholamine levels were measured radioenzymatically.[1,39] Plasma yohimbine and 11-hydroxy-yohimbine levels were quantitated by reverse-phase HPLC with fluorometric detection.[1,40] The within-day assay reproducibilities for yohimbine and 11-hydroxy-yohimbine were 3.8% and 5.9%, and the lower limits of detection were 0.1 and 1 ng/mL, respectively.[40]

Analysis of yohimbine, 10-hydroxy-yohimbine, and 11-hydroxy-yohimbine (Fig 1) in microsome experiments was performed by HPLC with a Waters system (model 600 gradient pump, WISP 717-plus automatic injector, model 996 photo diode array detector, and Millennium 32 data analysis software; Waters Corp, Milford, Mass). The residues from extraction were dissolved in 50 μL of initial mobile phase, and a 20-μL aliquot was injected onto a Supelco Discovery C-18 column (250 × 5 mm) (Sigma-Aldrich, St Louis, Mo) with the thermostat at 45°C in a Waters model 600 oven (Waters Corp). Initial conditions of the mobile phase (10% acetonitrile in 0.01-mol/L potasssium dihydrogen phosphate and 0.085% phosphoric acid, pH 2.2) were immediately followed by a linear gradient from 10% to 40% acetonitrile in 15 minutes. The final conditions were held for 2 minutes, after which the column was re-equilibrated for 8 minutes. Extracted channels at a wavelength of 221 nm from the photo diode array detector data were processed for area-under-peak determination. 10-Hydroxy-yohimbine, 11-hydroxy-yohimbine, and yohimbine eluted at 5.5, 6.2, and 13 minutes, respectively.

Metabolic pathway of yohimbine: Incubation conditions for microsomal oxidation

Yohimbine, at a substrate concentration from 1 to 250 μmol/L, was incubated in the presence of microsomes from cells containing heterologously expressed cytochrome P450 (CYP) isoforms coexpressed with CYP/nicotinamide adenine dinucleotide phosphate reductase. CYP2D6, CYP3A4, and CYP3A5, expressed in a baculovirus system (Gentest Corp, Woburn, Mass), were separated into aliquots, stored at −80°C, and thawed on ice before use. Microsomes were used at various protein concentrations (0.120, 0.116, and 0.118 mg/mL for CYP2D6, CYP3A4, and CYP3A5, respectively) in the presence of nicotinamide adenine dinucleotide phosphate (1.3 mmol/L), glucose-6-phosphate (3.3 mmol/L), magnesium chloride (3.3 mmol/L), and glucose-6-phosphate dehydrogenase (0.2 U) in a total volume of 0.5 mL of 100-mmol/L potassium phosphate (pH 7.4). In preliminary experiments, the time courses of metabolite formation were checked to define conditions of reaction linearity (5 to 40 minutes) and to ensure an overall substrate consumption lower than 10%. Reactions, performed in triplicate, were initiated by addition of cofactors, followed by incubation for 10 minutes at 37°C in a shaking water bath open to the atmosphere. Reactions were stopped by addition of methylene chloride (4 mL), and the mixture was immediately shaken for 5 minutes and then centrifuged. The organic layer (3.5 mL) was evaporated to dryness under a nitrogen stream.

Genotyping

Genomic deoxyribonucleic acid (DNA) was extracted from leukocytes in frozen, ethylenediaminetetraacetic acid–anticoagulated whole blood by use of a commercial kit (PureGene; Gentra Biosystems, Minneapolis, Minn); the typical yield was approximately 20 ng/μL blood.

CYP2D6

Genomic DNA was then used as a template for initial polymerase chain reaction (PCR) in preparation for assays of *CYP2D6* variants by allele-specific nested PCR or restriction fragment length polymorphism, as previously described.[41,42] For *CYP2D6*, the variant assays were wild type versus the less active (or inactive) variants **3*, **8*, **6*, **4*, **10*, or **17*.

CYP3A4

For *CYP3A4*, single nucleotide polymorphism variants were scored in a 2-stage assay.[43] In stage 1, PCR primers flanking the polymorphism were used to amplify the target region from 5 ng of genomic DNA. In stage 2, an oligonucleotide primer flanking the variant was annealed to the amplified template and extended across the variant base. The mass of the extension

product (wild type versus variant) was scored by matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF) mass spectrometry (low-mass allele versus high-mass allele). In this study, 2 different *CYP3A4* single-nucleotide polymorphisms were scored. *CYP3A4* variant A−290G is reported as a promoter polymorphism 290 base pairs (bp) upstream of the initiation codon (ATG) in *CYP3A4*[44]; in our subjects this site proved to be monomorphic (all A alleles). *CYP3A4* variant T16090C (rs2246709) is located at base pair position 16,090 downstream of the *CYP3A4* cap (transcription initiation) site; this position is within intron 7, 258 bp downstream from exon 7 (National Center for Biotechnology Information reference clone NT_007933.13). Because the final extension primer was in the antisense orientation, the bases incorporated across the variant position were G (major allele, 67%) versus A (minor allele, 33%). The 2 alleles were in Hardy-Weinberg equilibrium in this sample ($\chi^2 = 2.37$, $P = .124$).

CYP3A5

For *CYP3A5*, the variant assays were wild type (**1*) versus the less active variant **3*. *CYP3A5 *3* genotyping was performed by allele-specific PCR based on the method of Hiratsuka et al[45,46] with the modifications described here. We adapted the assay to a real-time SYBR (registered trademark of Molecular Probes, Inc, Eugene, Ore) Green assay by eliminating the fluorescent-labeled *Taq*Man probe and modifying the PCR mix. We used the Platinum Quantitative PCR Supermix-UDG (Invitrogen Corporation, Carlsbad, Calif), the common and allele-specific primers described by Hiratsuka et al (final concentration of 400 nmol/L), SYBR Green I (1:100,000 final dilution; Molecular Probes), fluorescein (final concentration of 1 nmol/L), and 10 to 30 ng of DNA template. Control DNA samples of known **1/*1*, **1/*3*, and **3/*3* genotypes were included in each run to facilitate genotype calling and as quality controls. Assays were conducted in a Bio-Rad iCycler (Biorad Laboratories, Hercules, Calif). The amplification detection protocol was as follows: 1 cycle at 50°C for 2 minutes, 1 cycle at 95°C for 5 minutes, 45 cycles at 95°C for 10 seconds and 60°C for 35 seconds, and a melt curve at the end of 80 cycles starting at 60°C and increasing by 0.5°C per cycle. Genotype determinations were made by comparing the threshold cycle (C_t) of the reaction containing the **1* primer versus that containing the **3* primer for each sample. There was consistently a difference of 7 C_t values between the perfect-matched and the mismatched primers in the homozygous samples, and the C_t values were similar for the heterozygous samples.

Data analyses

Initial (baseline, before drug) intergroup hemodynamic and biochemical measurements were compared by nonparametric Mann-Whitney *U* test. Group demographic data were compared by chi square test. Responses to yohimbine were assessed by 2-factor repeated-measures ANOVA.[47] Histograms of responses were plotted with the number of bins (intervals into which the data were divided) approximating the $\sqrt{n}$.[48] Distributions of responses were evaluated for number of admixed components by maximum likelihood analysis, by use of the program ADMIX for IBM-compatible microcomputers,[1,49] or by cluster analysis.[47] Results are expressed as mean value ± 1 SEM. A value of $P \leq .05$ defined statistical significance. Statistical analyses were undertaken in the program SPSS for Macintosh (version 10.0; SPSS Inc, Chicago, Ill).

Kinetic parameters (maximum rate of metabolism [V_{max}] and Michaelis-Menten constant [K_m]) were determined by nonlinear least squares regression analysis with Sigmaplot software (SPSS Inc), by use of the Michaelis-Menten equation or the Hill equation where appropriate.[50] Model selection was based on visual inspection of the goodness of fit and application of Akaike's information criterion.[51] Intrinsic clearance (CL_{int}) was determined as follows:

$$CL_{int} = V_{max}/K_m$$

A simulation of the contribution of the individual CYP isoforms to the formation of 10- and 11-hydroxy-yohimbine as a function of substrate concentration was performed,[52] based on the relative abundance of the isoforms in the human liver[53] and their kinetic parameters.

RESULTS

Yohimbine metabolism: Bimodality of plasma 11-hydroxy-yohimbine

Plasma yohimbine and its oxidation metabolite (11-hydroxy-yohimbine) (Fig 1) assays were obtained in 172 individuals, 15 minutes after initiation of the yohimbine intravenous infusion. There was an extremely broad range of yohimbine metabolism, spanning more than 3 orders of magnitude (Fig 2).

Seventeen individuals showed no detectable yohimbine metabolism (plasma 11-hydroxy-yohimbine concentration <1 μg/L [the lower limit of detection]).

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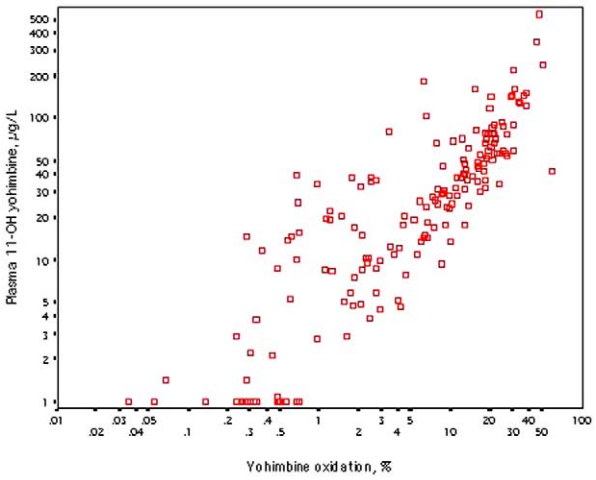

Fig 2. Distribution and range of yohimbine metabolism. Plasma 11-hydroxy (OH)-yohimbine is shown as a function of percent oxidation of yohimbine to that metabolite (11-OH-yohimbine/total yohimbine). Results obtained after 15 minutes of yohimbine intravenous infusion are shown for 172 individuals. Seventeen individuals had plasma 11-OH-yohimbine concentrations beneath the lower limit of detection (<1 µg/L); hence percent oxidation values in these 17 subjects represent upper bounds.

When these individuals were contrasted with the 155 subjects who did show detectable yohimbine oxidation, the 2 groups differed (Table I) in ethnicity ($P = .005$) but not in age, sex, body habitus, blood pressure, heart rate, or family history of hypertension. In 155 individuals in whom yohimbine was metabolized, 12.0% ± 0.91% of the circulating yohimbine was converted to 11-hydroxy-yohimbine by 15 minutes. The metabolizer groups also differed in plasma renin activity and leptin, perhaps consistently with the group differences in ethnicity.

The frequency histogram (Fig 3) of plasma 11-hydroxy-yohimbine concentration revealed a bimodal distribution, with 20 of 172 subjects (11.6%) in the interval with less than 1.4 µg/L 11-hydroxy-yohimbine. To avoid any artifactual appearance of bimodality as a result of skewed data, the data were $\log_{10}$-transformed and only approximately 13 intervals ($\sqrt{n}$) were plotted. Bimodality was confirmed by maximum likelihood analysis, which strongly favored bimodal over unimodal models for plasma 11-hydroxy-yohimbine concentration ($\chi^2 = 165.4$, $P < .001$). Two modes were also suggested by k-means cluster analysis ($F = 162$, $P < .001$).

Effect of ethnicity on yohimbine metabolism

A Kruskal-Wallis nonparametric ANOVA revealed ethnicity as a significant determinant of yohimbine metabolizer status ($\chi^2 = 8.89$, $df = 2$, $P = .012$), a result confirmed by likelihood ratio test ($\chi^2 = 9.04$, $df = 2$, $P = .011$) and standard Pearson chi square test ($\chi^2 = 8.94$, $df = 2$, $P = .011$), as well as parametric 1-way ANOVA ($F = 4.63$, $P = .011$). Post hoc tests on the 1-way ANOVA indicated that the white group had a greater frequency of nonmetabolizers than was found in the other groups (Tukey $P = .012$, Scheffé $P = .019$, Bonferroni $P = .015$). In the white group, 13 of 73 (17.8%) showed no oxidation of yohimbine, as compared with 3 of 74 (4.1%) in the black group and 1 of 25 (4.0%) in the other group (Fig 4).

We used a multivariate model (stepwise linear regression) to study which of several independent variables was the best predictor of yohimbine metabolizer status, including demographic (age, ethnicity, sex, family history of hypertension), physical (body surface area, body mass index), or physiologic (blood pressure status) variables. Among these variables, ethnicity was the sole significant predictor of yohimbine metabolizer status (multiple $R = 0.202$, $R^2 = 0.041$, adjusted $R^2 = 0.035$, $F = 7.20$, $t = 2.68$, $P = .008$).

Metabolic pathways of yohimbine in vitro

The kinetics of yohimbine microsomal hydroxylation to 10-hydroxy-yohimbine and 11-hydroxy-yohimbine (Fig 1) was best described by a 1-enzyme Michaelis-Menten (K_m model for each CYP. The kinetic parameters are listed in Table II. The CYP2D6 isoform had a much lower (ie, more favorable, by 9- to 245-fold) K_m value than was found in the CYP3A isoforms for both 10- and 11-hydroxylation of yohimbine.

The intrinsic clearance (CL_{int}) for the formation of 10-hydroxy-yohimbine was higher (more favorable) than that of 11-hydroxy-yohimbine for both CYP3A4 and CYP3A5, in contrast to CYP2D6, which preferentially formed 11-hydroxy-yohimbine (higher CL_{int}). Moreover, the CL_{int} value of CYP3A4 was much higher than that of CYP3A5 for both 10- and 11-hydroxylation of yohimbine. The CL_{int} rank order (ranging from most favorable to least favorable) of the CYP isoforms for the formation of 11-hydroxy-yohimbine was CYP2D6 > CYP3A4 > CYP3A5, whereas it was CYP3A4 > CYP2D6 > CYP3A5 for the formation of 10-hydroxy-yohimbine.

More precise information regarding the contribution of the individual CYP isoforms to the formation of 10- and 11-hydroxy-yohimbine, with their relative abun-

Table I. Characteristics of subject groups divided by presence or absence of detectable oxidation of yohimbine to 11-hydroxy-yohimbine

Variable	*11-Hydroxy-yohimbine formation*		
	Absent	*P value*	*Present*
No.	17		155
Demographic characteristic			
Age (y)	42 ± 2.8	.788	40 ± 1.1
Sex (men/women)	6/11	.221	79/76
Ethnicity (white/black/other)*	13/3/1	**.005**	60/71/24
Blood pressure status (hypertensive/normotensive)	5/12	.938	47/108
Family history of hypertension (+/–)	16/1	.990	146/9
Diabetes status (+/–)	1/16	.899	8/147
Physical			
Height (m)	1.69 ± 0.020	.963	1.69 ± 0.086
Weight (kg)	83.8 ± 6.0	.835	82.8 ± 1.5
Body surface area (m^2)	1.93 ± 0.068	.971	1.92 ± 0.021
Body mass index (kg/m^2)	29.2 ± 2.06	.756	28.9 ± 0.460
Physiologic			
Blood pressure (mm Hg)			
Systolic	135 ± 5.2	.469	132 ± 1.7
Mean	99 ± 3.7	.356	95 ± 1.3
Diastolic	80 ± 3.2	.321	77 ± 1.1
Pulse pressure	55 ± 3.0	.886	55 ± 1.0
Heart rate (beats/min)	68 ± 3.1	.534	66 ± 0.9
Glomerular filtration rate ($mL \cdot min^{-1} \cdot 1.73\ m^{-2}$)	105 ± 6.0	.440	109 ± 2.0
Biochemical			
Plasma			
Glucose (mg/dL)	93 ± 10.3	.534	89 ± 3.4
Creatinine (mg/dL)	0.88 ± 0.042	.590	0.90 ± 0.015
Cholesterol (mg/dL)	207 ± 12.5	.195	190 ± 3.7
Triglycerides (mg/dL)	106 ± 22.4	.867	104 ± 6.6
Leptin (ng/mL)	24.8 ± 4.7	**.013**	14.3 ± 1.1
Renin activity (ng angiotensin $I \cdot mL^{-1} \cdot h^{-1}$)	3.02 ± 0.582	**.030**	1.97 ± 0.145
Norepinephrine (pg/mL)	367 ± 39.9	.242	317 ± 11.5
Epinephrine (pg/mL)	18 ± 3.4	.124	25 ± 1.7
Dopamine (pg/mL)	26 ± 5.4	.463	23 ± 1.7
Urine			
Norepinephrine (μg/g creatinine)	34,368 ± 7237	.620	40,048 ± 2594
Epinephrine (μg/g creatinine)	12,634 ± 2685	.194	18,638 ± 1148
Yohimbine metabolism (peak plasma values at 15 min)			
11-Hydroxy-yohimbine (μg/L)	<1 (undetectable)	**<.001**	49.4 ± 5.13
Yohimbine (μg/L)	558 ± 177	.896	634 ± 71
% Oxidation (yohimbine to 11-hydroxy-yohimbine)	Undetectable	**<.001**	12.0 ± 0.912

Intergroup comparisons were performed by nonparametric tests (Mann-Whitney *U* or chi square), with any significant *P* values ($P < .05$) given in bold type in a column between the 2 groups.
Other includes Hispanic, Asian, Native American, and mixed.

dance in the human liver and their kinetic parameters taken into account, is given in Fig 5. On the basis of this simulation, in a yohimbine concentration range up to 500 μg/L, the contribution of CYP3A4 to the formation of 10-hydroxy-yohimbine is approximately 70% whereas the contribution of CYP2D6 to the formation of 11-hydroxy-yohimbine is approximately 80%.

CYP genotyping and yohimbine metabolism in vivo: *CYP2D6*, *CYP3A4*, and *CYP3A5*

When the CYP loci were analyzed individually, yohimbine metabolism in vivo could not be predicted by allelic variation at *CYP2D6* ($F = 0.221$, $P = .803$), *CYP3A4* ($F = 0.462$, $P = .632$), or *CYP3A5* ($F = 1.03$, $P = .360$).

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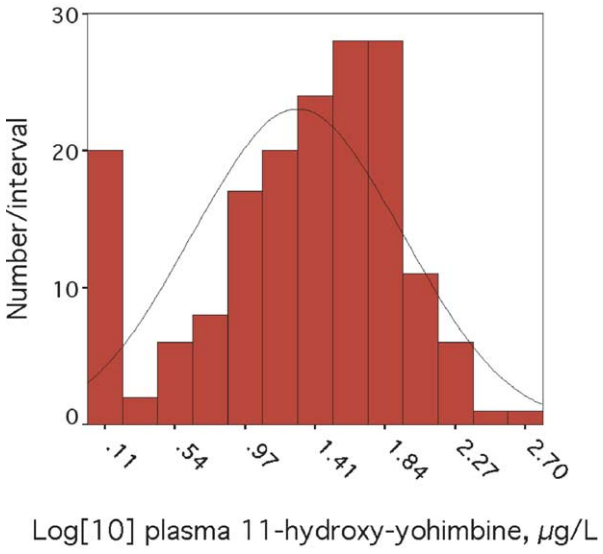

Fig 3. Frequency histogram of plasma 11-OH-yohimbine concentration. Results were obtained in 172 individuals after 15 minutes of intravenous yohimbine infusion. The number of intervals (bins) was set at the nearest integral number to $\sqrt{n} = \sqrt{172}$ = approximately 13. The apparent lower mode includes 20 individuals with very low (<1.4 μg/L; <0.146 on log scale) plasma 11-OH-yohimbine concentrations; 17 of these individuals had undetectable (<1 μg/L) concentrations of 11-OH-yohimbine. Bimodality: $\chi^2 = 41.5$, $df = 2$, $P < .001$.

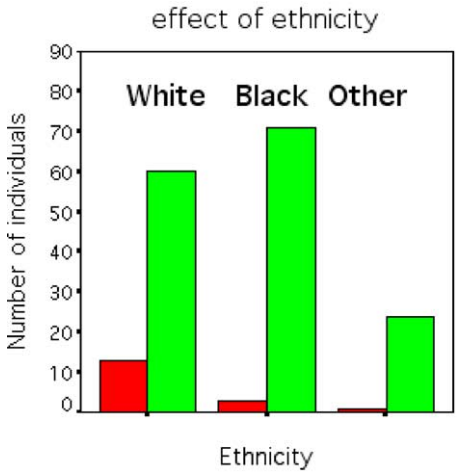

Fig 4. Yohimbine oxidation as a function of ethnicity. Subjects who did (n = 155) or did not (n = 17) have detectable yohimbine oxidation are grouped by ethnicity. *Black bars,* 11-OH-yohimbine absent; *gray bars,* 11-OH-yohimbine present. Seventeen individuals had undetectable (<1 μg/L) plasma 11-OH-yohimbine concentrations after 15 minutes of intravenous yohimbine. The number of individuals in each self-identified ethnic group was as follows: white, n = 73; black, n = 74; or other (Hispanic or Asian), n = 25. $\chi^2 = 8.94$, 2 *df*, $P = .011$.

However, by 2-way ANOVA (Fig 6), stratified genotypes at *CYP2D6* and *CYP3A4* jointly predicted the yohimbine percent conversion to the 11-hydroxy metabolite (overall F = 3.03, $P = .005$). The analysis revealed a significant interaction between *CYP2D6* and *CYP3A4* genotypes to predict 11-hydroxylation of yohimbine (interaction F = 3.05, $P = .033$), suggesting an epistatic (nonadditive or permissive) interaction of alleles at 2 different loci to influence the final phenotype. In this model, sex was a significant covariate (F = 10.3, $P = .002$). Incorporation of *CYP3A5* did not improve the model, and there was no apparent interaction of *CYP3A5* with either *CYP2D6* or *CYP3A4*.

At *CYP2D6* in 88 subjects, 28 (32%) were wild type, 50 (57%) were heterozygotic for inactivating mutations (1 allele; **3*, **8*, **6*, **4*, **10*, or **17*), and 10 (11%) had 2 inactivating mutations (both alleles).

At *CYP3A4* variant T16090C (antisense A16090G) in 67 subjects, 33 (49%) were wild-type homozygotes (G/G), 24 (36%) were heterozygotes (G/A), and 10 (15%) were homozygotes for the minor allele (A/A). The allele frequencies were 67% for A and 33% for G; the 2 alleles were in Hardy-Weinberg equilibrium ($\chi^2 = 2.37$, $P = .124$). *CYP3A4* variant A−290G was monomorphic (all A alleles) in each individual in this study.

For *CYP3A5* genotypes, 11 (12%) were wild type (**1/*1*), 37 (41%) were heterozygotes for inactivating mutations (**1/*3*), and 42 (47%) had 2 inactivating mutations (**3/*3*). The allele frequencies were 33% for **1* and 67% for **3*; the 2 alleles were in Hardy-Weinberg equilibrium ($\chi^2 = 0.405$, $P = .524$).

Physiologic and biochemical responses to α_2-adrenergic blockade: Effect of yohimbine metabolism

Results were analyzed by 2-way repeated-measures ANOVA to evaluate simultaneously the effects of drug (yohimbine), drug metabolizer status, and the interaction of the two (Table III).

Neurotransmitters. The yohimbine metabolizer groups differed in the plasma norepinephrine response to the drug (Table III). The level of plasma norepinephrine rose overall (F = 115, $P < .001$) but climbed approximately 33% higher (to 1087 ± 155 pg/mL versus 816 ± 40 pg/mL) in the nonmetabolizers (interaction F = 3.98, $P = .048$) (Fig 7). Perhaps because of ethnic differences in metabolizer frequency (Fig 4),

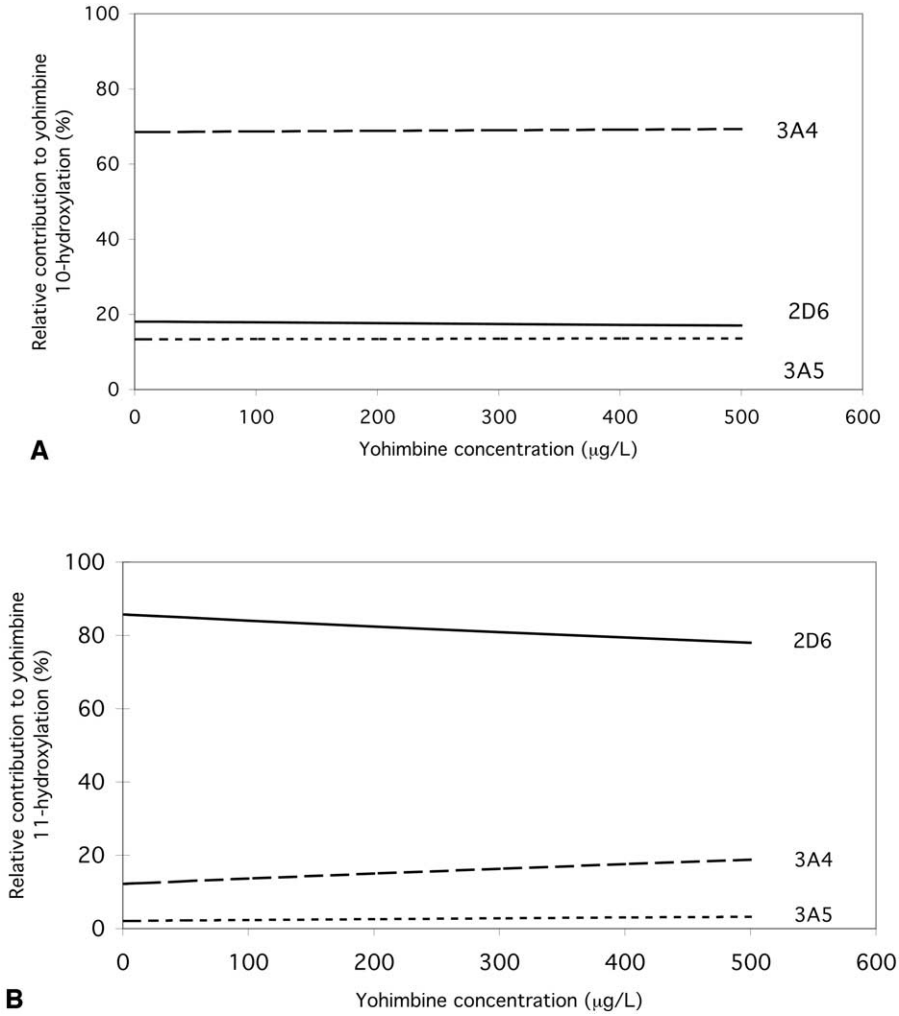

Fig 5. Simulation of relative contributions of CYP2D6, CYP3A4, and CYP3A5 to formation of 10-OH-yohimbine **(A)** and 11-OH-yohimbine **(B)**, as a function of substrate (yohimbine) concentration.

Table II. Metabolism of yohimbine by hepatic microsomes to its oxidative catabolites (10-hydroxy-yohimbine or 11-hydroxy-yohimbine)

Metabolic pathway		*V_{max} (pmol · min^{-1} · mg^{-2} microsomal protein)*		*K_m (μmol/L)*		*CL_{int} (μL · min^{-1} · mg^{-1} microsomal protein)*	*CL_{int} 10-OH/ 11-OH ratio*
CYP	*Product*	*Value*	*SEM*	*Value*	*SEM*		
CYP3A4	10-OH-YO	2870	219	150	24	19	1.8
	11-OH-YO	1898	187	181	34	10	
CYP3A5	10-OH-YO	344	128	826	378	0.4	2.1
	11-OH-YO	99	71	489	489	0.2	
CYP2D6	10-OH-YO	84.9	4.9	16.8	4.1	5.1	0.067
	11-OH-YO	147.4	6.1	2.0	0.5	75.5	

V_{max}, Maximal rate of conversion of yohimbine to metabolite at saturating concentration of yohimbine; K_m, concentration of yohimbine for half-maximal conversion to metabolite; CL_{int}, intrinsic clearance (V_{max}/K_m); 10-OH-YO, 10-hydroxy-yohimbine; 11-OH-YO, 11-hydroxy-yohimbine.

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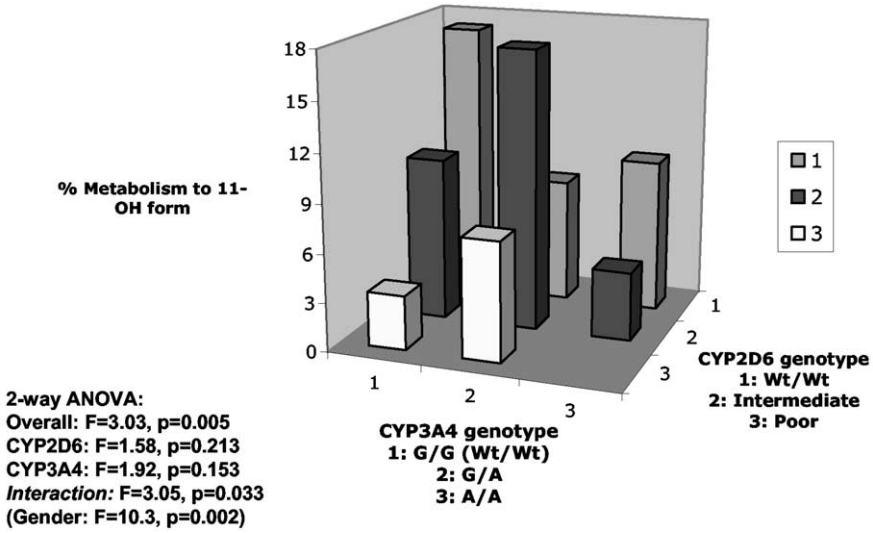

Fig 6. Cytochrome P450 (*CYP2D6* and *CYP3A4*) genotypes as predictors of yohimbine metabolism in vivo. Subjects were classified by the ability to metabolize yohimbine to detectable 11-OH-yohimbine as described in Fig 4. *CYP2D6* genotypes were classified by the number of alleles (0, 1, or 2) found to be inactivating variants (**3*, **8*, **6*, **4*, **10*, or **17*) in each subject; both wild-type (Wt) alleles predicted extensive metabolism, 1 inactivating variant predicted intermediate metabolism, and 2 inactivating variants predicted poor metabolism. *CYP3A4* genotypes (sense C16090T, antisense G16090A) were classified by major (wild-type) allele (G) versus minor allele (A) in each subject.

increments in norepinephrine level also tended to differ by ethnicity ($F = 2.99$, $P = .053$). Epinephrine level also rose overall ($F = 4.25$, $P = .041$), although the rise was not different in the 2 metabolizer groups ($F = 0.878$, $P = .350$); likewise, dopamine level rose after yohimbine administration ($F = 10.3$, $P = .002$) but without a difference in the metabolizer groups ($F = 0.542$, $P = .463$).

Hemodynamics. Yohimbine infusion significantly and substantially increased systolic ($F = 15.7$, $P < .001$), diastolic ($F = 33.2$, $P < .001$), and mean ($F = 27.7$, $P < .001$) blood pressures (Table III). The increment in blood pressure resulted from a rise in cardiac output ($F = 3.36$, $P = .010$) rather than systemic vascular resistance ($F = 2.03$, $P = .089$). In turn, the rise in cardiac output was accounted for by stroke volume ($F = 2.55$, $P = .038$) rather than heart rate ($F = 0.694$, $P = .427$). Hemodynamic responses to yohimbine did not differ by metabolizer group (all $P > .3$ for interaction).

DISCUSSION

Yohimbine is a well-characterized α_2-adrenergic antagonist and has, therefore, been used to probe the role of the α_2-adrenergic receptors, as well as postreceptor signaling pathways, in cardiovascular and neurologic disease.[2,29,30,32] Indeed, we have used yohimbine in this way to examine hereditary alterations in α_2-adrenergic function that are apparent even before the onset of high blood pressure in persons at genetic risk of essential hypertension.[1] Yohimbine also has several therapeutic uses in the treatment of impotence or erectile dysfunction, orthostatic hypotension in autonomic failure, xerostomia (dry mouth), narcolepsy, sensorineural deafness, and depression.[32,33]

However, the ultimate effect of any drug in vivo reflects the influences of not only pharmacodynamics (what is the action of the drug once it binds at its target site?) but also pharmacokinetics (what events affect the arrival of the drug at its receptor?).[54]

Bimodality of yohimbine metabolism

Yohimbine is metabolized by oxidation to both 11-hydroxy-yohimbine and 10-hydroxy-yohimbine (Fig 1).[34] Hydroxylation of drugs typically increases their solubility, thereby promoting faster excretion.[50] The inactive 10-hydroxy metabolite is highly water-soluble, rapidly excreted, and typically detected in urine but not plasma.[34] The 11-hydroxy metabolite retains biologic activity for some effects (on platelet aggregation and

Table III. Hemodynamic and biochemical responses to α_2-adrenergic blockade: Stratification by time and by yohimbine metabolism status

Variable	*Yohimbine oxidation: Absent (n = 17) versus present (n = 155)*	*Time after intravenous yohimbine*				
		0 min	*5 min*	*10 min*	*15 min*	*20 min*
Physiologic						
Blood pressure (mm Hg)						
Systolic	–	146 ± 4.6	149 ± 5.8	155 ± 6.5	158 ± 5.9	160 ± 6.7
	+	144 ± 1.8	150 ± 2.2	156 ± 2.1	156 ± 2.2	156 ± 2.2
Diastolic	–	84 ± 3.6	86 ± 3.4	91 ± 3.9	93 ± 4.0	94 ± 4.5
	+	80 ± 1.0	84 ± 1.1	88 ± 1.1	89 ± 1.1	91 ± 1.1
Mean	–	105 ± 3.4	107 ± 4.1	112 ± 4.5	115 ± 4.3	116 ± 5.0
	+	101 ± 1.2	106 ± 1.3	111 ± 1.3	111 ± 1.4	112 ± 1.4
Systemic vascular resistance, (mm Hg · L^{-1} · min^{-1})	–	17.8 ± 1.03	17.9 ± 1.3	18.7 ± 1.6	18.6 ± 1.6	19.6 ± 1.6
	+	18.4 ± 0.48	18.2 ± 0.49	18.4 ± 0.47	18.7 ± 0.54	18.5 ± 0.50
Cardiac output (L/min)	–	6.12 ± 0.31	6.45 ± 0.55	6.45 ± 0.41	6.68 ± 0.48	6.39 ± 0.47
	+	5.94 ± 0.15	6.24 ± 0.13	6.49 ± 0.14	6.54 ± 0.18	6.56 ± 0.14
Heart rate (beats/min)	–	69 ± 3.4	69 ± 2.9	67 ± 3.0	68 ± 3.4	67 ± 3.3
	+	65 ± 1.0	68 ± 1.0	67 ± 1.1	67 ± 1.1	68 ± 1.1
Stroke volume (mL/beat)	–	93 ± 7.6	96 ± 8.8	100 ± 8.7	101 ± 9.3	99 ± 9.5
	+	94 ± 2.6	93 ± 2.1	97 ± 2.4	99 ± 2.9	100 ± 2.5
Biochemical (plasma)						
Norepinephrine (pg/mL)	–	367 ± 40	—	—	1087 ± 155	—
	+	322 ± 12	—	—	816 ± 40	—
Epinephrine (pg/mL)	–	17 ± 3.2	—	—	25 ± 5.0	—
	+	30 ± 2.3	—	—	37 ± 4.4	—
Dopamine (pg/mL)	–	26 ± 5.4	—	—	37 ± 7.8	—
	+	23 ± 1.7	—	—	32 ± 2.4	—
Yohimbine (μg/L)	–	—	—	—	558 ± 177	—
	+	—	—	—	634 ± 71	—
11-Hydroxy-yohimbine (μg/L)	–	—	—	—	<1	—
	+	—	—	—	49.4 ± 5.13	—

F and *P* values were calculated by 2-factor analysis of variance with repeated measures at times 0, 5, 10, 15, and 20 minutes. Bold type denotes $P \leq .05$. Blood pressures were obtained continuously by Finapres (see Methods). Yohimbine and 11-hydroxy-yohimbine differences were evaluated by the nonparametric Mann-Whitney *U* test.

lipolysis) in vitro, although its access to the α_2-adrenergic receptor may be affected by its lower albumin binding: The free fraction of 11-hydroxy-yohimbine, at 57%, is higher than that of yohimbine itself, at 18%.[55]

Here, we measured both yohimbine and its 11-hydroxylated metabolite levels[34,40] to probe disposition of the drug in human subjects. Even though yohimbine was administered intravenously (thus bypassing such pharmacokinetic influences as variability in systemic bioavailability through differences in absorption or first-pass hepatic metabolism), we found that yohimbine metabolism was highly variable: Over a 15-minute time course, the degree of oxidation of yohimbine to 11-hydroxy-yohimbine varied more than 1000-fold (Fig 2), with 17 of 152 individuals showing no detectable oxidation. The possibility that yohimbine metabolism is a discrete trait is raised by the bimodal distribution of plasma 11-hydroxy-yohimbine concentration (Fig 3), which suggests that yohimbine metabolism is a discrete trait; evidence for bimodality was supported by maximum likelihood analysis ($\chi^2 = 165.4$, $P < .001$) and cluster analysis ($F = 162$, $P < .001$).

Lack of ability to oxidize yohimbine was distributed nonrandomly across ethnic strata: The trait was most common (at approximately 18%; $\chi^2 = 8.89$, $P = .012$) in individuals of European ancestry (Fig 4). What underlies the interindividual variability in yohimbine oxidation? Yohimbine is initially oxidized to 11-hydroxy and 10-hydroxy forms,[34,40] but the precise

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2-Factor repeated-measures ANOVA		
Effect of yohimbine	*Yohimbine oxidation status*	*Yohimbine × oxidation interaction*
F = 15.7, **P < 0.001**	F = 0.048, P = 0.828	F = 0.36, P = 0.837
F = 33.2, **P < 0.001**	F = 0.901, P = 0.344	F = 0.65, P = 0.627
F = 27.7, **P < 0.001**	F = 0.425, P = 0.515	F = 0.491, P = 0.742
F = 2.03, **P = 0.089**	F = 1.14, P = 0.335	F < 0.001, P = 0.970
F = 3.36, **P = 0.010**	F = 0.022, P = 0.881	F = 0.430, P = 0.787
F = 0.694, P = 0.427	F = 0.071, P = 0.791	F = 1.42, P = 0.226
F = 2.55, **P = 0.038**	F = 0.017, P = 0.897	F = 0.179, P = 0.949
F = 115, **P < 0.001**	F = 3.98, **P = 0.048**	F = 4.02, **P = 0.047**
F = 4.25, **P = 0.041**	F = 0.878, P = 0.350	F = 0.0, P = 0.995
F = 10.3, **P = 0.002**	F = 0.542, P = 0.463	F = 0.142, P = 0.707
—	P = 0.896	—
—	**P < 0.001**	—

metabolizing enzyme was not previously well established.

The microsomal oxidation experiments (Table II, Fig 5) showed that CYP2D6 and CYP3A isoforms had quite different roles in hydroxylation of yohimbine in vitro, displaying region selectivity for the 11- and 10-positions. Indeed, CYP3A isoforms had CL_{int} values for the formation of 10-hydroxy-yohimbine that were twice those for the formation of 11-hydroxy-yohimbine, whereas CYP2D6 preferentially catalyzed the formation of 11-hydroxy-yohimbine, with an 11-hydroxy-yohimbine/10-hydroxy-yohimbine CL_{int} ratio of approximately 15:1. When the relative abundance of these CYP isoforms in the human liver was taken into account,[51] CYP2D6 and CYP3A4 were initially predicted to be the main isoforms involved in hydroxylation of yohimbine.

In addition to oxidation of the α_2-adrenergic antagonist yohimbine (Table II, Fig 5), metabolism of the β-adrenergic antagonists alprenolol, metoprolol, timolol, and propranolol, as well as other drugs active on the cardiovascular system, is the responsibility of CYP2D6.[56,57] The substantial contribution of CYP2D6 to the in vitro 11-hydroxylation of yohimbine (Table II, Fig 5) is in agreement with previous in vivo data showing, in a subject who was a poor metabolizer of debrisoquin (INN, debrisoquine) (an index of CYP2D6 activity[56]), that the systemic clearance of yohimbine was greatly diminished, as assessed after intravenous dosing and, to a lesser extent, oral dosing.[34] Our simulation of the relative contributions of different CYP isoforms to the in vitro hydroxylation of yohimbine (Fig 5) cannot necessarily be extrapolated to oral dosing of yohimbine, because of the potential role of intestinal or liver metabolism,[51] perhaps involving CYP3A4, CYP3A5, or other CYP isoforms.

Indeed, genotyping at the *CYP2D6* and *CYP3A4* loci indicated that both of these enzymes seem to be important for disposition of yohimbine in vivo (Fig 6). By 2-way ANOVA, a model incorporating both *CYP2D6* and *CYP3A4* genotypes predicted metabolism ($F = 3.03$, $P = .005$). Although neither locus alone was significant (both $P > .05$), an interaction of the 2 loci was apparent in determination of the phenotype ($F = 3.05$, $P = .033$). Genes on 2 different chromosomes encode these cytochromes—*CYP2D6* on chromosome 22q13 and *CYP3A4* on chromosome 7. Such permissive (nonadditive) interaction between genes in determining a phenotype is compatible with the genetic concept of epistasis.

Bimodality and ethnic variability are hallmarks of oxidative metabolism of other drugs, most commonly in CYP-catalyzed oxidations. Indeed, some inactivating mutations of *CYP2D6* are especially common in individuals of European ancestry,[52,53] the subgroup in our study most likely to show slowed metabolism of yohimbine (Fig 4). Other CYP isoforms also have ethnic variations in activity: Up to approximately one half of all drugs are oxidatively metabolized by the CYP isoform CYP3A, a major component of whose activity is CYP3A5; inactivating mutations of *CYP3A5* are especially common in white subjects, in whom the liver typically expresses only approximately one half as much CYP3A5 as is expressed in black subjects.[58]

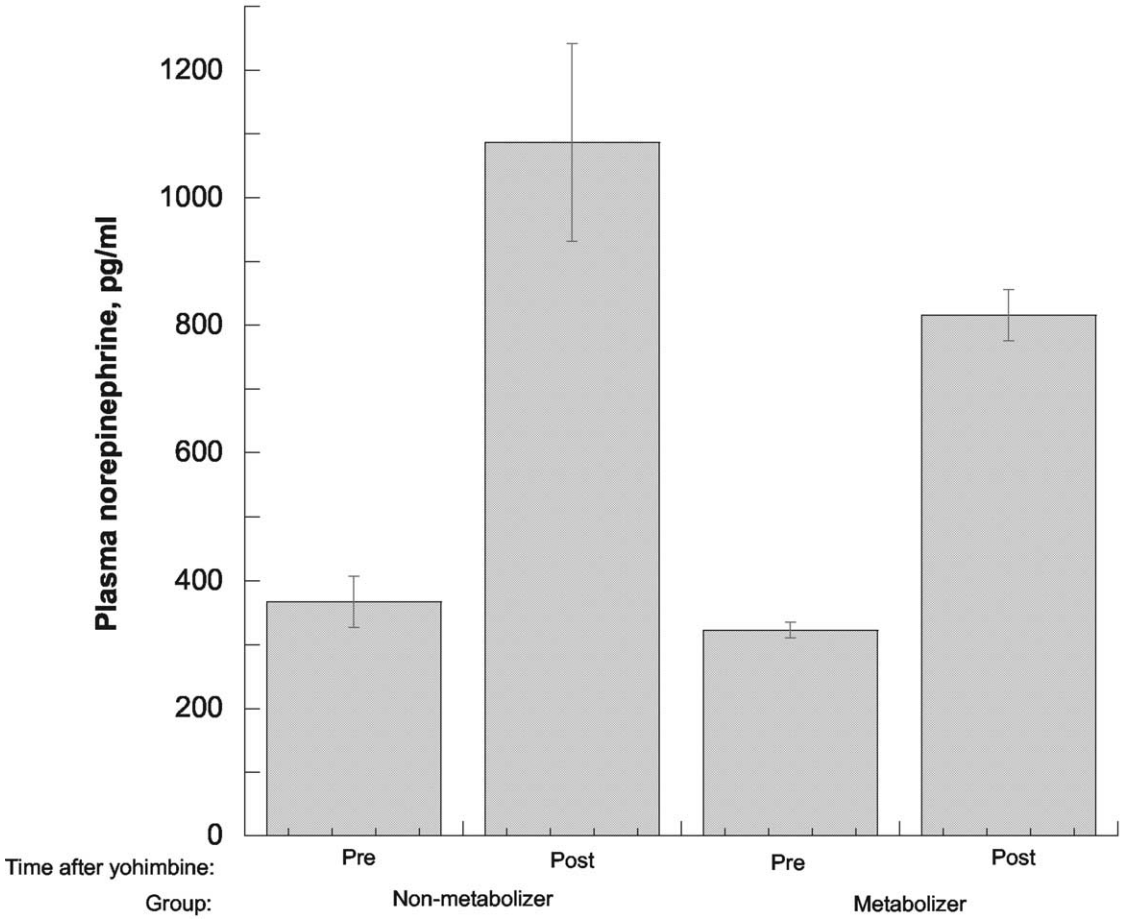

Fig 7. Plasma norepinephrine responses to α_2-adrenergic blockade with yohimbine in subjects stratified by ability to oxidize the drug to 11-OH-yohimbine, nonmetabolizers (n = 17) versus metabolizers (n = 155). Results were analyzed by 2-way repeated-measures ANOVA as follows: drug, F = 115.4, $P < .001$; metabolizer group, F = 3.98, $P = .048$; drug × metabolizer group interaction, F = 4.02, $P = .047$.

Sympathetic neuronal consequences of bimodal yohimbine metabolism

Yohimbine elevates blood pressure by acting on central α_2-adrenergic receptors to increase efferent sympathetic outflow,[1,2,29,30,59-63] leading to increased blood pressure by elevating cardiac output, which in turn results from increased stroke volume rather than heart rate (Table III).

Our subjects showed increments in all 3 plasma catecholamine levels after yohimbine, as follows: norepinephrine (F = 115, $P < .001$), epinephrine (F = 4.25, $P = .041$), and dopamine (F = 10.3, $P = .002$). However, poor yohimbine metabolizers displayed an approximately 33% greater increment in norepinephrine level (F = 3.98, $P = .041$; Fig 7) but no differential increases in epinephrine or dopamine level. The selective effect of yohimbine metabolizer status on norepinephrine (rather than epinephrine or dopamine) suggests that local inactivation of yohimbine may be more influential on sympathetic neuronal rather than adrenal medullary function. This suggestion is reinforced by the similar plasma concentrations of active, untransformed yohimbine in the 2 metabolizer groups (Table I).

Grasing et al[64] reported that plasma yohimbine's area under the time-concentration curve predicted interindividual differences in autonomic responses to the drug. Although we did not note differences in plasma yohimbine concentration in the metabolizer groups ($P = .896$, Table III), we only measured

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this concentration at 1 time point (15 minutes into infusion) and were, therefore, unable to assess the integrated effect of altered metabolism on the organism. Sampling only plasma might also miss alterations of active drug concentration at such critical sites as sympathetic nerve terminals[1,2,13,16,18,20,21,24-26,63] or baroreceptor nuclei in the brain stem.[14,61]

Conclusions and implications

The α_2-adrenergic antagonist yohimbine is a powerful trigger to sympathetic nervous system activation, raising stroke volume and cardiac output and thereby elevating blood pressure.

There is a very broad range of oxidative metabolism of the compound to 11-hydroxy-yohimbine, with a bimodal distribution of the metabolite in the bloodstream, and overrepresentation of nonmetabolizers among persons of European ancestry. Microsomal oxidation studies indicated that the major CYP metabolizing yohimbine to 11-hydroxy-yohimbine in vitro is likely CYP2D6 (with the lowest K_m and highest CL_{int}), whereas in vivo both *CYP2D6* and *CYP3A4* genotypes apparently interacted in an epistatic fashion to influence metabolizer phenotype. Nonmetabolizers showed exaggerated norepinephrine release after yohimbine, indicating that pharmacokinetic factors affect the sympathetic neuronal response to α_2-adrenergic blockade.

These results illustrate the importance of precise pathways of drug metabolism for adrenergic outcomes and thus have implications for both diagnostic and therapeutic uses of this α_2-adrenergic antagonist. Diagnostically, investigators have used the autonomic response to yohimbine as a clue to genetic predisposition to hypertension[1,2] or in rodent experimental models.[28] Our results indicate that it would be prudent to consider genetic variability in drug metabolism as an additional hereditary component of the autonomic response to yohimbine. Yohimbine metabolizer status clearly influenced patterns of norepinephrine release (Fig 7) after the drug, although hemodynamic responses were not affected (Table III).

Therapeutic use of yohimbine typically consists of the orally administered compound.[22,32] Although our diagnostic study used intravenous yohimbine, the same CYP polymorphisms (*CYP2D6* and *CYP3A4*) would be expected to govern metabolism of the drug after oral administration. Thus we would predict that individuals with inactivating mutations at both the *CYP2D6* and *CYP3A4* loci (Fig 6) might be especially susceptible to the autonomic effects of the drug. However, after oral administration, initial ("first-pass") metabolism by the small intestine and liver is likely to be especially important; in those tissues the relatively high local abundance of such cytochromes as CYP3A4 and CYP3A5 could be crucial.[53] Future studies of plasma yohimbine and its metabolites, coupled with appropriately targeted CYP genotyping (*CYP2D6*, *CYP3A4*, and *CYP3A5*) (Fig 5, Table II), are likely to yield profiles to guide oral treatment with the drug.

None of the authors have conflicts of interest (any financial and personal relationships that could potentially be perceived as influencing the described research).

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